JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1981, p. 355-360
0095-1137/81/100355-06$02.00/0

Vol. 14, No. 4

Bacteriological and Serological Studies of *Haemophilus equigenitalis*, Agent of Contagious Equine Metritis

DANIEL J. TAINTURIER,[1]* CATHERINE F. DELMAS,[2] AND HENRY J. DABERNAT[2]

Service de Pathologie de la Reproduction, Ecole Nationale Vétérinaire, 31076 Toulouse,[1] and Laboratoire de Bactériologie-Virologie, Faculté de Médecine Purpan, 31400 Toulouse, France[2]

Received 9 December 1980/Accepted 21 May 1981

Seventeen strains of *Haemophilus equigenitalis* isolated from the cervix, clitoris, and urethra of mares were biochemically characterized with the API 10E and API ZYM test kit systems, conventional biochemical tests, and the porphyrin test. Antisera were prepared in rabbits. All of the strains were positive to the porphyrin test, and the requirement for factor X (hemin) or V (nicotinamide adenine dinucleotide) was not shown. Catalase, oxidase, phosphatase, and phosphoamidase tests were positive with all of the strains. Aminopeptidase (arylamidase) activity has been detected on beta-naphthylamide derivatives of eight amino acids and of di- and tripeptides. No glycosidase activity was found. Antisera prepared in rabbits strongly agglutinated all *H. equigenitalis* strains, but none of the various other bacterial strains. These characteristics should prove to be useful in the identification of *H. equigenitalis*.

In 1977, a new venereal disease appeared in horses in Great Britain (15, 16). In 1976, the same disease had been noted in Ireland (13), the causative agent remaining unidentified. It has also been found in France (10, 14), Australia (4), the United States (20), and West Germany (12). Although transmission is venereal, it is clinically manifested only in the mare. The agent responsible for contagious equine metritis has been isolated from the genital tract of mares (cervix, vagina, clitoris, and urethra) and from the external genitalia of stallions. The recovered mare and the contaminated stallion may remain chronic carriers.

The causative agent is a fastidious gram-negative, microaerophilic, encapsulated (19), nonfermentative coccobacillus, growing in 3 days on chocolate agar incubated under 5 to 10% CO_2. The organism is very unreactive in biochemical tests; only oxidase, catalase, and phosphatase tests are positive. In 1978, Taylor et al. (22) proposed that the causative organism of the contagious equine metritis be considered a new species of *Haemophilus* named *Haemophilus equigenitalis*. Although some stimulation by factor X (hemin) was observed, a strict dependence upon factor X or V (nicotinamide adenine dinucleotide) was not demonstrated (18, 22).

Since Taylor et al. (22) described the type strain, no extensive study has been undertaken concerning this new species. Without prejudice to its unclear taxonomic position (24), we used conventional tests and enzymatic test kit systems to obtain as broad a picture as possible of the biochemical activity of these bacteria. In addition, *H. equigenitalis* antisera prepared in rabbits were evaluated for bacterial identification.

MATERIALS AND METHODS

Bacterial strains. Seventeen strains of *H. equigenitalis* were examined in the present investigation, including the reference strain NCTC 11184. They are listed in Table 1. Strains were stored freeze-dried or frozen at −70°C in brain heart infusion broth (Difco Laboratories) containing 15% (vol/vol) glycerol. In addition, fresh clinical isolates of various bacterial species were subjected to the same biochemical test or slide agglutination test or both.

Growth conditions, colony morphology, and bacterial morphology. Comparative studies of growth were done with various agar media: chocolate agar supplemented with 1% IsoVitaleX (Bio-Mérieux), chocolate agar (10% [vol/vol] heated defibrinated horse, sheep, or rabbit blood in Eugon agar [Bio-Mérieux]), Mueller-Hinton agar (Difco), brain heart infusion agar (Difco), beef-yeast extract agar (Institut Pasteur), tryptic soy agar (Difco) with or without 1% Fildes (Difco) or IsoVitaleX, and unheated blood agar (10% vol/vol defibrinated horse or sheep blood in blood agar base [Difco]).

Brain heart infusion broth (Difco) with and without 1% Fildes and peptone water (Institut Pasteur) were used as liquid media.

The inoculated media were incubated at 37°C in air in an anaerobic atmosphere (GasPak, BBL Microbiology Systems) or in 5 to 10% CO_2 (candle jar system or GasPak without catalyst).

Bacterial morphology was determined with cells

TABLE 1. *H. equigenitalis strains*

Strain designation[a]	Year of isolation	Source
001	1978	Cervix
002	1977	Cervix
003	1978	Clitoris
004	1978	Cervix
005	1978	Urethra
006	1978	Cervix
007	1978	Cervix
008	1978	Cervix
009	1978	Clitoris
010	1978	Urethra
011	1978	Clitoris
012	1978	Cervix
013	1978	Cervix
014	1977	Cervix
015	Unknown	Unknown
016	Unknown	Unknown
017	1980	Cervix

[a] All of the strains were obtained from J. Pitre, Laboratoire Départemental et Régional de Biologie et d'Hygiène, Caen, Calvados, France, with the exception of: 002, from P. Gaumont, Laboratoire Central de Recherches Vétérinaires, Maisons-Alfort, France; 014, from the National Collection of Type Cultures (NCTC) (11184, Collection de l'Institut Pasteur [CIP] 7907); 015, from C. Tram, Institut Pasteur, Paris; and 016, NCTC 11225, CIP 7944.

from chocolate agar and cells from brain heart infusion broth incubated at 37°C for 72 h.

API ZYM test. Various API ZYM test kits were used: API ZYM (19 tests), API esterase (10 tests), API aminopeptidase (54 tests), and API transpeptidase (2 tests) (API system S.A., La Balme les Grottes, Montalieu Vercieu, France). The substrates consisted of naphthyl derivatives of fatty acids and carbohydrates and of β-naphthylamide or *p*-nitroanilide derivatives of tetra-, tri-, and dipeptides and of amino acids. The API ZYM strips were inoculated according to the directions of the manufacturer with 2 drops of a heavy bacterial suspension in distilled water (turbidity, 5 to 6 on the McFarland scale) obtained from bacterial growth on an agar plate. The strips were incubated in moist chambers for 18 h at 37°C and were read according to the directions of the manufacturer.

This series of tests was applied to all 17 strains of *H. equigenitalis* and to fresh clinical isolates of *H. influenzae*, *H. parainfluenzae*, *Brucella abortus*, *B. melitensis*, *Pasteurella multocida*, and *Acinetobacter calcoaceticus*.

Each strain was examined at least twice to determine the reproducibility of the results.

API 10E. Test kits were inoculated according to the instructions of the manufacturer and were used for the study of ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase, urease, and indole production. The strips were incubated for 18 h at 37°C. Reagents were added when necessary, and the reactions were read according to the recommendations of the manufacturer. Urease and indole production were also studied in Urée-Indole medium (Institut Pasteur).

Porphyrin test. The synthesis of porphyrins from Δ-amino-levulinic acid was investigated according to Kilian (6).

X and V factor requirement. The requirement for X and V factors was investigated with X-, V-, and X and V-containing disks (Difco) on brain heart infusion agar.

Oxidase. The presence of an oxidase was investigated with 72-h growth, and disks were impregnated with dimethyl-*p*-phenylenediamine (Bio-Mérieux).

Catalase. The production of a catalase was investigated with colonies removed from an agar culture and placed in 1 drop of 10% (vol/vol) H_2O_2 on a glass slide.

***o*-Nitrophenyl-β-D-galactopyranoside test.** The *o*-nitrophenyl-β-D-galactopyranoside tests were performed with a dense suspension of bacteria in saline (0.85% NaCl) and disks containing *o*-nitrophenyl-β-D-galactopyranoside (Bio-Mérieux).

Antisera. Strains 001 and NCTC 11184 were used to prepare *H. equigenitalis* antisera in rabbits. Bacterial growth on a chocolate agar plate (Eugon agar base [Bio-Mérieux] with 10% horse blood heated to 80°C for 10 min) obtained after 72 h at 37°C in 10% CO_2 was transferred to saline (0.85% NaCl), washed twice in saline, resuspended in saline, and heated to 56°C for 75 min. The optical density of the 10-fold-diluted suspension was 0.77 at 380 nm. After a preimmune bleeding, two groups of New Zealand white nongravid does were inoculated subcutaneously five times over a 4-week period. The inoculations were three 2-ml and two 1-ml doses for the first group and two 0.5-ml doses with 0.5 ml of a complete Freund adjuvant suspension and three 1-ml doses for the second group. Blood was taken by cardiac puncture 7 to 29 days after the last injection and allowed to clot. Sera were separated and stored at −70°C.

Titration of antisera. A tube agglutination technique was used for the titration of antisera with the above suspension of bacteria as antigen. None of the animals had agglutinating antibodies before the first injection. Antibody titers varied from 160 to 1,280 after the vaccination. Only the sera presenting high titers (≥640) were used for slide agglutination tests.

Slide agglutination tests. Slide agglutination tests were performed with prepared antisera undiluted or diluted to 1/2, 1/5, and 1/10 and with specific *B. abortus*, *B. melitensis* (Bio Mérieux), and *H. influenzae* (Difco) antisera.

One to two colonies were mixed homogeneously with 1 drop of saline, and then 1 drop of antiserum was added. The slides were gently rotated by hand for 2 min before the mixture was examined for agglutination. The strains tested against *H. equigenitalis* antisera are listed in Table 3.

RESULTS

Growth characteristics. Optimal growth was observed at 37°C in an atmosphere with 5 to 10% CO_2 on chocolate agar and supplemented media. The best growth was observed on chocolate agar (10% [vol/vol] heated, defibrinated horse blood in Eugon agar). After 72 h of incu-

TABLE 2. *Biochemical characteristics of 17 strains of H. equigenitalis and strains of other bacterial species*

Characteristic	*H. equigenitalis*[a] NCTC 11184	*H. equigenitalis*[b] (17 strains)	*H. influenzae*[a]	*H. parainfluenzae*[a]	*B. abortus*[a]	*B. melitensis*[a]	*P. multocida*[a]	*A. calcoaceticus*[a]
Catalase	+	+	+	+	+	+	+	+
Cytochrome oxidase	+	+	+	+	+	+	+	−
Porphyrin test	+	+	−	+	+	+	+	+
o-Nitrophenyl-β-D-galactopyranoside test	−	−	−	−	−	−	−	−
Urease	−	−	+	+	+	+	−	−
Indole production	−	−	+	−	−	−	+	−
Ornithine decarboxylase	−	−	+	−	−	−	−	−
Lysine decarboxylase	−	−	+	−	−	−	−	−
Arginine dihydrolase	−	−	−	+	−	−	−	−
Phosphatase	+	+	+	+	+	+	+	+
Phosphoamidase	+	+	+	+	−	−	+	−
Glycine arylamidase	+	+	+	−	+	+	+	−
L-Alanine arylamidase	+	+	+	−	+	+	+	+
L-Serine arylamidase	+	+	−	−	+	+	−	−
Methionine arylamidase	+	+	−	−	−	−	−	−
L-Aspartate arylamidase	+	+	−	−	−	−	−	−
α-L-Glutamate arylamidase	+	+	−	−	−	−	−	−
L-Lysine arylamidase	+	+	−	−	−	−	−	−
L-Arginine arylamidase	+	+	+	−	+	+	−	−
L-Alanyl-L-alanine arylamidase	+	+	+	−	+	+	+	+
L-Alanyl-L-alanyl-L-alanine arylamidase	+	+	+	−	+	+	+	+
L-Glutamine arylamidase	+	+	−	−	−	−	−	−
L-Leucyl-L-alanine arylamidase	+	+	−	−	+	+	+	−
Glycyl glycine arylamidase	+	+	−	−	−	−	−	−
Glycyl phenylalanine arylamidase	+	+	−	−	−	−	−	−
Leucyl glycine arylamidase	+	+	−	−	+	+	−	−
Glycyl-L-alanine arylamidase	+	+	+	+	+	+	+	+
L-Lysyl-L-alanine arylamidase	+	+	−	−	−	−	−	−
L-Phenylalanyl-L-prolyl-L-alanine arylamidase	+	+	−	−	−	−	−	−
L-Seryl-L-methionine arylamidase	+	+	−	−	−	−	−	−
L-Phenylalanine arylamidase	+	+	−	−	−	−	−	−
L-Phenylalanyl-L-arginine arylamidase	−	+	−	−	−	−	−	−
L-Prolyl-L-ariginine arylamidase	+	+	−	−	+	+	−	−
L-Leucine arylamidase	+	+	−	−	−	−	−	−
L-Seryl tyrosine arylamidase	+	d	−	−	+	+	−	−
α-L-Aspartyl-L-alanine arylamidase	+	d	−	−	−	−	−	−
L-Lysyl-L-lysine arylamidase	−	d	−	−	−	−	−	−

TABLE 2. —*Continued*

Characteristic	*H. equigenitalis*[a] NCTC 11184	*H. equigenitalis*[b] (17 strains)	*H. influenzae*[a]	*H. parainfluenzae*[a]	*B. abortus*[a]	*B. melitensis*[a]	*P. multocida*[a]	*A. calcoaceticus*[a]
S-benzyl cysteine arylamidase	−	d	−	−	−	−	−	−
L-Tryptophane arylamidase	−	d	−	−	−	−	−	−
L-Arginyl-L-arginine arylamidase	−	d	−	−	−	−	−	−
L-Ornithine arylamidase	−	d	−	−	+	+	−	−
L-Alanyl-L-phenylalanyl-L-prolyl-L-alanine arylamidase	−	d	−	−	−	−	−	−
γ-L-glutamate transpeptidase	−	d	−	−	−	−	−	−
Glycyl-L-tryptophane arylamidase	−	d	−	−	−	−	−	−
L-Histidine arylamidase	−	±	−	−	+	+	−	−
α-L-Glutamyl-α-L-glutamic arylamidase	−	±	−	−	−	−	−	−
Glycyl-L-arginine arylamidase	−	±	−	−	−	−	−	−
L-Tyrosine arylamidase	−	±	−	−	−	−	−	−
L-Proline arylamidase	−	±	−	−	+	+	−	−
D-Alanine arylamidase	−	−	+	−	+	+	−	−
NCBZ glycyl glycyl-L-arginine arylamidase	−	−	−	−	−	+	−	−
Esterase (4-C)	−	d	+	+	+	+	+	+
Esterase (5-C)	+	+	+	−	+	+	−	+
Esterase (6-C)	+	+	+	−	+	+	−	+
Esterase (8-C)	+	+	+	−	+	+	+	+
Esterase (9-C)	−	±	−	−	+	+	+	+

[a] +, Positive characteristic for the strain tested; −, negative characteristic for the strain tested.

[b] +, More than 88% positive reactions; −, more than 88% negative reactions; d, 47 to 87% positive reactions; ±, 13 to 46% positive reactions.

bation, colonies were smooth, convex, greyish-white, opaque, with entire edges, odorless, and 1 to 1.5 mm in diameter. On unheated blood agar and on nutrient agar, a film of growth was seen after prolonged incubation (5 days or more). In deeply inoculated beef-yeast extract agar, the growth appeared only in a disk-shaped zone 2 to 6 mm from the top of the medium. In Gram smears from agar culture, the bacteria exhibited mostly a coccobacillary form. However, a few rod shapes were observed in smears from liquid media.

No stimulation of growth around X-, V-, and X and V-containing disks was observed on brain heart infusion agar. Furthermore, the porphyrin test was positive for all strains of *H. equigenitalis*.

Biochemical characteristics. All strains of *H. equigenitalis* were reproducibly positive in the catalase, cytochrome oxidase, phosphatase, and phosphoamidase tests, and all were reproducibly negative in the urease, indole, ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase, and *o*-nitrophenyl-β-D-galactopyranoside tests. They were also negative for α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosaminidase, α-mannosidase, and α-fucosidase. The results concerning the other substrates are listed in Table 2.

Slide agglutination tests. Both antisera were tested against the strains of *H. equigenitalis* and strains of various species of bacteria. The diluted and undiluted *H. equigenitalis* antisera strongly agglutinated the homologous and heterologous *H. equigenitalis* strains, but did not react with any of the other species tested (Table 3). No cross-reactions were observed between *Brucella* and *Haemophilus* antisera and the *H. equigenitalis* strains.

DISCUSSION

In this report, we present the results of investigations of 17 strains of *H. equigenitalis*. In 1978, Taylor et al. (22) described the causative

TABLE 3. *Slide agglutination tests with H. equigenitalis antisera*

Antigen[a]	Agglutination with antiserum at given dilution							
	H. equigenitalis NCTC 11184				*H. equigenitalis* 001 France			
	Undiluted	1/2	1/5	1/10	Undiluted	1/2	1/5	1/10
Haemophilus equigenitalis NCTC 11184	+	+	+	+	+	+	+	+
Haemophilus equigenitalis NCTC 11125	+	+	+	+	+	+	+	+
Haemophilus equigenitalis (other strains)	+	+	+	+	+	+	+	+
H. influenzae	−	−	−	−	−	−	−	−
H. parainfluenzae	−	−	−	−	−	−	−	−
Brucella abortus	−	−	−	−	−	−	−	−
B. melitensis	−	−	−	−	−	−	−	−
Pasteurella multocida	−	−	−	−	−	−	−	−
Yersinia enterocolitica	−	−	−	−	−	−	−	−
Y. pseudotuberculosis	−	−	−	−	−	−	−	−
Klebsiella pneumoniae	−	−	−	−	−	−	−	−
Acinetobacter calcoaceticus	−	−	−	−	−	−	−	−
Pseudomonas aeruginosa	−	−	−	−	−	−	−	−
P. stutzeri	−	−	−	−	−	−	−	−
P. maltophilia	−	−	−	−	−	−	−	−
P. putida	−	−	−	−	−	−	−	−
P. multivorans	−	−	−	−	−	−	−	−

[a] Strains of *Pseudomonas fluorescens*, *Moraxella lacunata*, and *Aeromonas hydrophila* were not tested because they were self-agglutinating.

organism of contagious equine metritis. They determined its cultural and positive biochemical characteristics. The small number of the latter makes identification difficult. Although the bacterium does not require factor X or V, the authors considered it a new species of the genus *Haemophilus* instead of creating, for a single species, a new genus (22). In this study, we confirm the results obtained by several authors (14, 19, 22).

Micromethods investigating enzymatic capacities of bacteria on various substrates in the absence of bacterial growth have been used successfully in the study of various bacterial species: enterobacteria (9), staphylococci, streptococci and gram-negative bacilli (5), gram-negative anaerobes (23), *Actinomycetaceae* and related bacteria (8), and *Haemophilus* (3, 7).

Constant characteristics of *H. equigenitalis* were revealed by use of the 85 substrates as mentioned above. The strains tested do not possess glycosidases. On the other hand, aminopeptidase (arylamidase) activity has been found with β-naphthylamide derivatives of eight amino acids (glycine, L-alanine, L-serine, methionine, L-aspartate, L-glutamate, L-lysine, and L-arginine) and derivatives of di- and tripeptides. These characteristics were observed with all of the strains tested. They may be added to those defining the type strain. They allow an easier differentiation between the *H. equigenitalis* strains and the other species studied. Some characteristics among the others could be used with strains of various geographical origin in epidemiological studies.

Rabbit *H. equigenitalis* antisera were shown to be specific for this species. Also, they do not agglutinate strains of various other bacterial species. Utilization of these antisera will be useful in identifying suspect bacterial colonies obtained from specimens collected from mares or stallions. Isolation of *H. equigenitalis* is facilitated by the use of streptomycin in the culture medium (2, 11, 15, 17, 21, 22). However, a few strains are sensitive to streptomycin (1, 12, 14, 20), making their isolation difficult in some cases. These antisera, bound to a fluorescent compound, could be used directly on fresh specimens for the identification of *H. equigenitalis*. However, it is necessary to study the possible interactions between the labeled serum and various pathogenic or saprophytic bacterial species encountered in the genital tract of the mare (cervix, vagina, clitoris) and the external genitalia of the stallion. This study is in progress.

ACKNOWLEDGMENTS

We are very grateful to J. P. Gayral, Laboratoire de Recherche API, La Balme-les-Grottes, Montalieu Vercieu,

France, who has graciously contributed the materials for this study. We thank J. Pitre, R. Gaumont, and C. Tram for the gift of *H. equigenitalis* strains and C. Dion, M. Seguy, and M. F. Blanc for their technical assistance.

This work was supported in part by grant CA 1102, 1979 from the Institut National de la Recherche Agronomique—Haras.

LITERATURE CITED

1. **Dabernat, H. J., C. F. Delmas, D. J. Tainturier, and M. B. Lareng.** 1980. In vitro susceptibility of *Haemophilus equigenitalis*, the causative organism of contagious equine metritis 1977, to antimicrobial agents. Antimicrob. Agents Chemother. **18**:841–843.
2. **Gaumont, R.** 1979. Sur le diagnostic expérimental de la métrite contagieuse 1977 de la jument. Prat. Vét. Equine **11**:5–9.
3. **Hansen, W., E. Schoutens, and E. Yourassowsky.** 1979. Significance of the detection of beta-galactosidase and of beta-xylosidase in the taxonomic study of the genus *Haemophilus*. J. Clin. Pathol. **32**:1058–1060.
4. **Hughes, K. L., J. D. Bryden, and F. MacDonald.** 1978. Equine contagious metritis. Aust. Vet. J. **54**:101.
5. **Humble, M. W., A. King, and I. Phillips.** 1977. API ZYM: a simple rapid system for the detection of bacterial enzymes. J. Clin. Pathol. **30**:275–277.
6. **Kilian, M.** 1974. A rapid method for the differentiation of *Haemophilus* strains; the porphyrin test. Acta Pathol. Microbiol. Scand. Sect. B **82**:835–842.
7. **Kilian, M.** 1976. A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. J. Gen. Microbiol. **93**:9–62.
8. **Kilian, M.** 1978. Rapid identification of *Actinomycetaceae* and related bacteria. J. Clin. Microbiol. **8**:127–133.
9. **Kilian, M., and P. Bülow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B **84**:245–251.
10. **Lignieres, R.** 1980. Observations sur l'apparition de quelques foyers de métrite contagieuse équine en France. Bull. Acad. Vét. Fr. **53**:323–332.
11. **MacKintosh, M. E.** 1981. Bacteriological techniques in the diagnosis of equine genital infections. Vet. Rec. **108**: 52–55.
12. **Mumme, J., and R. Ahlswede.** 1978. Nachweis von *Haemophilus equigenitalis* im Zervixtupfer einer Warmblutstute. Dtsch. Tieraerztl. Wochenschr. **86**: 257–259.
13. **O'Driscoll, J. G., P. T. Troy, and F. J. Geoghegan.** 1977. An epidemic of venereal infection in thoroughbreds. Vet. Rec. **101**:359–360.
14. **Pitre, J., M. F. Legendre, and G. Voisin.** 1979. Informations et réflexions sur le diagnostic microbiologique et sérologique de la métrite coccobacillaire des équidés. Prat. Vét. Equine **11**:11–26.
15. **Platt, H., J. G. Atherton, D. J. Simpson, C. E. D. Taylor, R. O. Rosenthal, D. F. J. Brown, and T. G. Wreghitt.** 1977. Genital infection in mares. Vet. Rec. **101**:20.
16. **Powell, D. G.** 1978. Contagious equine metritis. Equine Vet. J. **10**:1–4.
17. **Sahu, S. P., and A. H. Dardiri.** 1980. Contagious equine metritis: isolation and characterization of the etiologic agent. Am. J. Vet. Res. **41**:1379–1382.
18. **Shreeve, J. E.** 1978. Preliminary observations on X-factor growth requirement of the bacterium responsible for CEM. Vet. Rec. **102**:20.
19. **Swaney, L. M., and S. S. Breese, Jr.** 1980. Ultrastructure of *Haemophilus equigenitalis*, causative agent of contagious equine metritis. Am. J. Vet. Res. **41**:127–132.
20. **Swerczek, T. W.** 1978. The first occurrence of contagious equine metritis in the United States. J. Am. Vet. Med. Assoc. **173**:405–407.
21. **Tainturier, D., T. Badin de Montjoie, H. J. Dabernat, and J. Ferney.** 1981. Etude expérimentale de la métrite contagieuse de la jument. Rev. Med. Vet. **132**:165–171.
22. **Taylor, C. E. D., R. O. Rosenthal, D. F. J. Brown, S. P. Lapace, L. R. Hill, and R. M. Legros.** 1978. The causative organism of contagious equine metritis 1977: proposal for a new species to be known as *Haemophilus equigenitalis*. Equine Vet. J. **10**:136–144.
23. **Tharagonnet, D., P. R. Sisson, C. M. Roxby, H. R. Ingham, and J. B. Selkon.** 1977. The API ZYM system in the identification of gram-negative anaerobes. J. Clin. Pathol. **30**:505–509.
24. **Zinnemann, K.** 1980. Newer knowledge in classification, taxonomy and pathogenicity of species in the genus *Haemophilus*. A critical review. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. A **247**:248–258.